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New vitamin D analogs

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Background. 1,25-$(OH)_2D_3$ (calcitriol) controls parathyroid gland growth and suppresses the synthesis and secretion of parathyroid hormone. Because of this, 1,25-$(OH)_2D_3$ has been used successfully for the treatment of secondary hyperparathyroidism, which almost always accompanies renal failure. However, the potent effect of 1,25-$(OH)_2D_3$ on intestinal calcium and phosphorus absorption and bone mineral mobilization often leads to the development of hypercalcemia and hyperphosphatemia precluding 1,25-$(OH)_2D_3$ therapy.

Methods. This has led to the development of vitamin D analogs that retain the suppressive action on PTH and parathyroid gland growth, but that have less calcemic and phosphatemic activity. Currently, two analogs, 19-nor-1,25-$(OH)_2D_2$ and 1,α(OH)D_2, are being used for the treatment of secondary hyperparathyroidism in the United States, and two are being used in Japan, 22-oxa-calcitriol and 1,25-$(OH)_2$-26,27F6 D_3.

Results. All four analogs suppressed PTH, but had less calcemic and phosphatemic activity than 1,25-$(OH)_2D_3$. In rats, 19-nor-1,25-$(OH)_2D_2$ has been shown to be less calcemic and phosphatemic compared to 1,α(OH)D_2.

Conclusion. Therapeutic doses of 19-nor-1,25-$(OH)_2D_2$ could produce a lower Ca x P product compared to 1,α(OH)D_2, which could be an important consideration in patient treatment. Further studies are necessary to define these differences and to understand the mechanisms behind the differential actions of vitamin D analogs.

Secondary hyperparathyroidism (HPT), a universal complication of renal failure, is characterized by enlarged parathyroid glands and an increase in parathyroid hormone (PTH) synthesis and secretion. Key factors in the development of SH are abnormalities in 1,25-$(OH)_2D_3$ production and metabolism, a decrease in the number of vitamin D receptors (VDR), and a resistance to the actions of 1,25-$(OH)_2D_3$, all of which occur as renal failure progresses. 1,25-$(OH)_2D_3$, the most active metabolite of vitamin D, controls parathyroid gland growth and suppresses the synthesis and secretion of PTH [1–4]. Because of its effect on PTH, 1,25-$(OH)_2D_3$ has been successfully used for the treatment of SH [5–8]. The potent effects of 1,25-$(OH)_2D_3$ on intestinal calcium absorption and bone calcium mobilization, however, can lead to hypercalcemia, often precluding 1,25-$(OH)_2D_3$ therapy. Hyperphosphatemia is also a persistent problem in these patients and can only be aggravated by the use of 1,25-$(OH)_2D_3$ [9, 10]. In addition, the use of calcium salts as phosphate binders only increases the risk of the development of hypercalcemia during 1,25-$(OH)_2D_3$ treatment [11–12].

The risk of hypercalcemia, as well as hyperphosphatemia brought on by 1,25-$(OH)_2D_3$ therapy, spurred the development of less calcemic and phosphatemic analogs of 1,25-$(OH)_2D_3$. The ideal analog for the treatment of SH would be at least as potent as 1,25-$(OH)_2D_3$ in suppressing PTH, but would have a minimal effect on calcium and phosphorus metabolism.

The biological actions of 1,25-$(OH)_2D_3$ are mediated through the vitamin D receptor (VDR). The binding of 1,25-$(OH)_2D_3$ to the VDR results in a conformational change in the VDR which allows it to also bind to the retinoid X receptor (RXR). The binding of this VDR/RXR complex to a specific sequence in the vitamin D-responsive element (VDRE) increases or decreases gene transcription.

Since there is evidence for only one form of nuclear VDR, it is assumed that the classic actions (calcemic) and nonclassic actions (potentially therapeutic) are both mediated through the same VDR. One intriguing aspect of the newly developed analogs is their differential action in vivo compared to1,25-$(OH)_2D_3$. It would be expected that these new analogs would mimic the actions of 1,25-$(OH)_2D_3$ since most have a high affinity for the VDR, but their novel feature is their ability to support some, but not all, of the actions of 1,25-$(OH)_2D_3$. While they are potent suppressors of PTH, the analogs usually show a decreased potency in the intestine and bone, which results in decreased calcemic and phosphatemic responses. This selectivity has also been shown with vitamin D analogs developed for other therapeutic uses, such as psoriasis, immunomodulation, leukemia, and other cancers [13, 14]. Interestingly, the selectivity of these analogs is not always cell- or tissue-specific, but can be gene- or process-specific within the same tissue.

The relationship of the structure of a vitamin D com-

Key words: uremia, hyperparathyroidism, calcium, phosphorus, cacitriol.

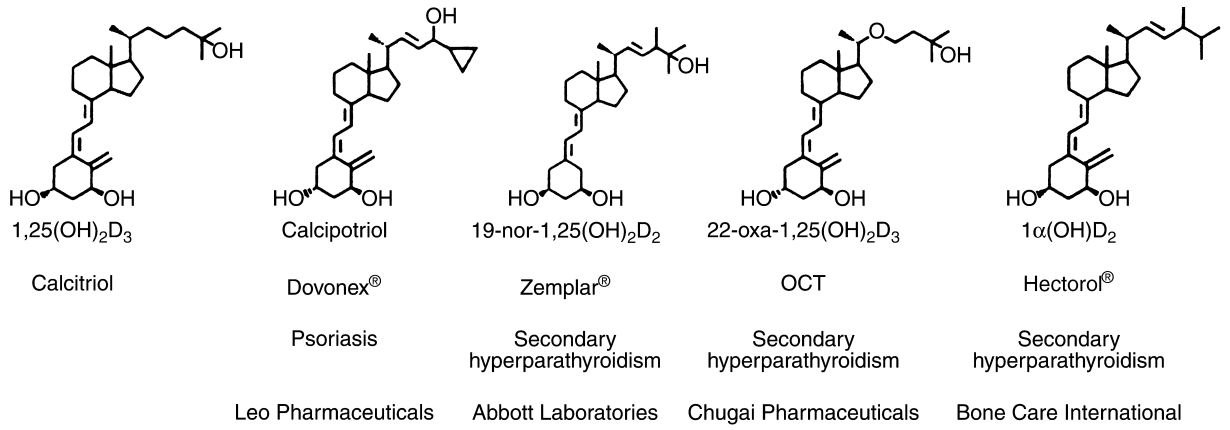

Fig. 1. Chemical structure of $1,25(OH)_2D_3$ and several vitamin D analogs. (Reproduced from reference [29]).

pound to its activity has been studied in detail [15]. Most critical for binding to the VDR is the A-ring, especially in the hydroxyl groups. While modifications in the D-ring or the side chain have little effect on VDR binding, they can affect biologic activity by altering the pharmacokinetics or catabolism of the compound. Analogs can also produce distinct conformational changes in the VDR that may produce gene-specific actions. Thus, a combination of structural modifications can produce analogs with diverse biologic profiles.

Selective vitamin D analogs for the treatment of SH

There are several requirements for vitamin D analogs developed for the treatment of SH. First, the analog has to have a reasonable affinity for the VDR. As earlier noted, this requires the presence of a hydroxyl group in the 1α position. Second, the analog needs to be substantially less calcemic than the parent compound. Despite a high affinity for the VDR, many analogs have significantly less calcemic activity than $1,25\text{-}(OH)_2D_3$. An example of this is $19\text{-nor-}1,25\text{-}(OH)_2D_2$, which is approximately 10 times less calcemic than $1,25\text{-}(OH)_2D_3$. The decreased calcemic activity of $19\text{-nor-}1,25\text{-}(OH)_2D_2$ cannot be attributed to decreased VDR binding. Finally, the analog has to be able to suppress PTH in vivo. Although some analogs were effective in suppressing PTH in vitro, when tested in vivo, they were rapidly metabolized and not effective in treating SH. Four analogs are currently used for the treatment of SH, $19\text{-nor-}1,25\text{-}(OH)_2D_2$ (paricalcitol, Zemplar) and $1\alpha\text{-}(OH)D_2$ (doxercalciferol, Hectorol), in the United States, and 22-oxa-calcitriol (OCT) and $1,25\text{-}(OH)_2\text{-}26,27F6\ D_3$ (falecalcitriol), in Japan. The chemical structures of the first three compounds are shown in Figure 1.

We have extensively studied $19\text{-nor-}1,25\text{-}(OH)_2D_2$. As with all vitamin D_2 compounds, this analog has a carbon 28 and a double bond at the carbon 22 position. However, unlike all natural vitamin D compounds, it lacks the carbon 19 and the exocyclic double bond. In early studies, we found that $19\text{-nor-}1,25\text{-}(OH)_2D_2$ had the same potency as $1,25\text{-}(OH)_2D_3$ in suppressing PTH secretion in primary cultures of bovine parathyroid cells and could suppress pre-pro-PTH messenger RNA and PTH secretion without inducing hypercalcemia or hyperphosphatemia [16]. When given daily to parathyroidectomized rats fed either a calcium- or phosphorus-deficient diet for nine days, $19\text{-nor-}1,25\text{-}(OH)_2D_2$ generated smaller increases in plasma calcium and phosphorus than did $1,25\text{-}(OH)_2D_3$ [17]. In fact, additional dose-response studies showed $19\text{-nor-}1,25\text{-}(OH)_2D_2$ to be about 10 times less potent in mobilizing calcium and phosphorus from bone than $1,25\text{-}(OH)_2D_3$. Moreover, in contrast to $1,25\text{-}(OH)_2D_3$, which upregulates intestinal VDR, $19\text{-nor-}1,25\text{-}(OH)_2D_2$ had the opposite effect [18]. In an early trial in renal patients (Fig. 2), $19\text{-nor-}1,25\text{-}(OH)_2D_2$ was given at a graded dose of 0.04 μg/kg to 0.12 μg/kg over a seven-week period [19]. At the end of the study, plasma PTH levels had decreased an average of 60%. In addition, only seven incidences of hypercalcemia occurred out of 441 determinations. In each of these incidences PTH levels were decreased to less than 100 pg/mL, presumably as a result of the hypercalcemia. Thus, hypercalcemia was likely due to an excessive dose of $19\text{-nor-}1,25\text{-}(OH)_2D_2$. Since it is recommended that plasma PTH levels be maintained between 200 and 300 pg/mL to maintain normal bone histology, a lower dose of $19\text{-nor-}1,25\text{-}(OH)_2D_2$ could have been given, minimizing the likelihood of further hypercalcemic episodes. More recent metabolic studies in rats comparing $19\text{-nor-}1,25\text{-}(OH)_2D_2$ and $1\alpha(OH)D_2$ showed $19\text{-nor-}1,25\text{-}(OH)_2D_2$ to be less calcemic and phosphatemic than $1,\alpha(OH)D_2$ [20]. While both compounds suppressed PTH, therapeutic doses of $19\text{-nor-}1,25\text{-}(OH)_2D_2$ resulted in a lower Ca x P product compared to $1,\alpha(OH)D_2$. With our increasing understanding of the deleterious effects of vascular calcification on mortality and morbidity in the dialysis popula-

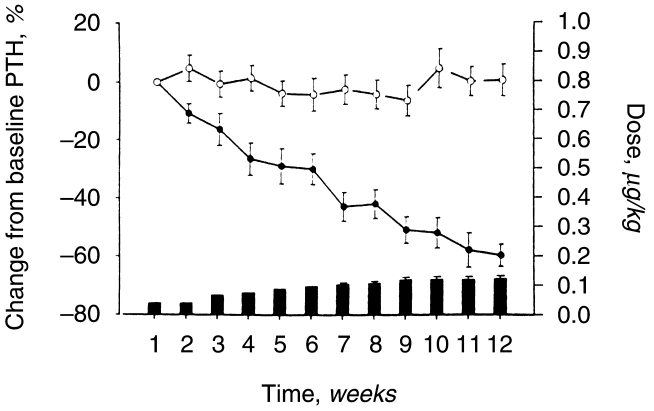

Fig. 2. Changes in the levels of intact PTH expressed as the percentage of the change from baseline values during the study period in placebo-treated (○) and 19-nor-1,25(OH)$_2$D$_2$-treated (●) patients. The bars depict the doses of 19-nor-1,25(OH)$_2$D$_2$ that increase according to the protocol. (Reproduced from reference [19]).

tion, this could be an important consideration in patient treatment.

1α(OH)D$_2$ is a pro-hormone and must be converted in the liver to its active metabolite, 1,25(OH)$_2$D$_2$. While this compound is less calcemic than 1,25-(OH)$_2$D$_3$, the basis for this is not well understood. While early studies comparing 1α(OH)D$_2$ and 1α(OH)D$_3$ showed 1α(OH)D$_2$ to be less toxic, the two compounds displayed the same potency in intestinal calcium transport and bone calcium mobilization [21, 22]. The effectiveness of 1α(OH)D$_2$ administered both orally and intravenously (IV) in the treatment of SH has been demonstrated [23, 24]. Intravenous administration of 1α(OH)D$_2$ produced smaller increments in serum calcium and phosphorus than oral dosing [23]. However, the prevalence of hypercalcemia and hyperphosphatemia were still high with IV therapy.

22-Oxacalcitriol, or OCT, differs from 1,25-(OH)$_2$D$_3$ only by the substitution of an oxygen atom in place of the carbon 22 in the side chain. Compared to 1,25-(OH)$_2$D$_3$, the affinity of OCT for the VDR and vitamin D-binding protein (DBP) are 8 and 400 to 500 times less, respectively. This alone could account for its lower activity in the intestine and bone. OCT is cleared rapidly from the circulation, which is probably secondary to its low DBP affinity. Previous studies demonstrated that a short exposure of the parathyroid glands to OCT leads to a prolonged suppression of PTH, while the stimulation of intestinal calcium absorption and bone resorption is short lived [25]. This could explain why OCT can suppress PTH yet have decreased calcemic and phosphatemic activities.

1,25-(OH)$_2$26,27F6 D$_3$, or falecalcitriol, is an analog in which carbons 26 and 27 are replaced by fluorine atoms. This analog is metabolized more slowly than 1,25-(OH)$_2$D$_3$, which accounts for its higher activity in vivo [26, 27].

Mechanisms for the differential actions of 1,25-(OH)$_2$D$_3$ and its analogs

The possible steps in the vitamin D pathway where differences in vitamin D analog action could lead to selective activities in vivo are shown in Fig. 3. The first of these is DBP affinity. The major carrier of vitamin D compounds in the circulation is the serum DBP. A decreased affinity for the DBP results in the analog being cleared faster from the circulation and thus, a shorter half-life. While this may explain the differential action of OCT, since its affinity for the DBP is 400 to 500 times less than that of 1,25-(OH)$_2$D$_3$, it does not explain that of 19-nor-1,25-(OH)$_2$D$_2$ or 1,25-(OH)$_2$D$_2$, the active metabolite of 1α(OH)D$_2$, since their affinities for the DBP are similar to 1,25-(OH)$_2$D$_3$.

In addition to enhancing the circulating half-life of vitamin D compounds, the DBP also decreases the tissue accessibility of 1,25-(OH)$_2$D$_3$. In this way, DBP helps to protect against vitamin D intoxication. Analogs with a decreased affinity for the DBP, although more quickly cleared from the circulation, may have increased tissue uptake. This could result in tissue-specific responses by increasing the response in tissues that require only a short exposure for a sustained response, while having little effect on tissues requiring long-term exposure to a vitamin D compound in order to sustain an effect.

Besides DBP, other proteins in circulation such as albumin and lipoproteins may bind small amounts of natural vitamin D compounds, but with lower affinity. While 99% of 1,25-(OH)$_2$D$_3$ in circulation is protein bound, mostly to DBP, other proteins may play a role in transporting analogs that have less affinity for the DBP. The impact, if any, of this on the differential actions of vitamin D analogs is not known.

Target cell metabolism plays an important role in several steroid hormone systems. Vitamin D compounds are primarily metabolized by the vitamin D-24-hydroxylase. Modifications in the side chains of some analogs may result in a different rate of catabolism, resulting in longer or shorter exposures of the analog to the tissue, or may result in different metabolites which may affect vitamin D action. Indeed, both of these have been shown to account, at least in part, for the unique properties of several vitamin D analogs.

A decreased affinity for the VDR may cause a decreased or blunted response by a vitamin D analog compared to the parent compound. While this may account in part for the differential actions of OCT, whose affinity for the VDR is about eight times less than that of 1,25-(OH)$_2$D$_3$, again, it does not explain the disparate actions of 19-nor-1,25-(OH)$_2$D$_2$ or 1,25-(OH)$_2$D$_2$, since their affinities for the VDR are similar to 1,25-(OH)$_2$D$_3$.

Upon binding to the VDR, 1,25-(OH)$_2$D$_3$ produces a specific conformational change in the VDR that allows

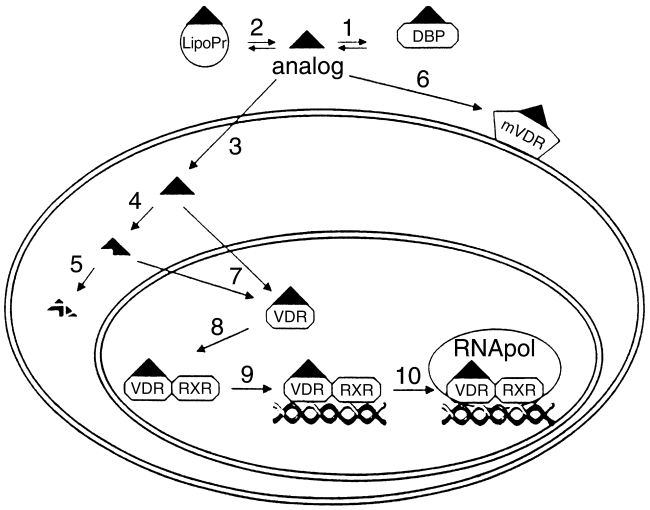

Fig. 3. Potential sites of differential action of $1,25(OH)_2D_3$ and its analogs. Possible points in the vitamin D activation pathway at which differences in vitamin D analog action could lead to selective activities in vivo are shown. The steps diagrammed include: (1) DBP affinity; (2) interaction with other serum proteins, including lipoproteins; (3) cellular uptake; (4) conversion to active metabolites; (5) catabolic inactivation; (6) activation of the nongenomic pathway through a putative membrane vitamin D receptor (mVDR); (7) interaction with the nuclear vitamin D receptor (VDR); (8) formation of the VDR/RXR complex; (9) binding to the activated complex to DNA; and (10) formation of the preinitiation complex with RNA polymerase II. (Reproduced from reference [29].)

it to also bind with RXR. The VDR/RXR complex then binds to a specific sequence in the target gene promoter called the vitamin D responsive element (VDRE), which results in an increase or decrease in gene transcription. Analogs may produce distinct conformational changes of their own which could alter the binding of RXR, resulting in either a weaker or stronger interaction with the VDRE.

Finally, once the VDR/RXR heterodimer is bound to the VDRE, it recruits other components of the transcriptional initiation complex. Differential recruitment of coactivators or corepressors to this complex can also play an important role on vitamin D analog transcription-induced biologic actions. Takeyema et al [28] demonstrated that calcitriol can induce the binding of several coactivators to the VDR that may enhance transcriptional activation, whereas OCT recruits only a subset of these. This could potentially produce biologic effects distinct from those of $1,25\text{-}(OH)_2D_3$ [29].

CONCLUSION

The vitamin D analogs currently used for the treatment of secondary hyperparathyroidism have less calcemic and phosphatemic activity while still effectively suppressing PTH. While pharmacokinetics and lower DBP binding may play a role in the selective activity of OCT compared to $1,25\text{-}(OH)_2D_3$, little is known about the mechanisms behind the selective actions of 19-nor-$1,25\text{-}(OH)_2D_2$ or $1,\alpha(OH)_2$. Better understanding of these mechanisms could provide insight for the design and development of more effective analogs in the future.

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